# LASER-ACTIVATED PROTEIN DEPOSITION FOR FABRICATION OF BIO-CHEMICAL FLUORESCENT SENSORS

Çağdaş Varel, Karl F. Böhringer University of Washington, Seattle, WA, USA



Figure 1: (a) Schematic of the setup used for laser-activated protein deposition. (b) Biotin binding after photobleaching of biotin-4-fluorescein (B4F). (c) Biotin-streptavidin binding after streptavidin addition. (d) Biotinylated anti-IgG binding to the deposited biotin. (e) Fluorescently labeled IgG is captured by the deposited anti-IgG.

#### ABSTRACT

This paper presents a fabrication method for biochemical sensors employing laser-activated protein deposition and reports the realization of an immunoglobulin G (IgG) sensor. It enables formation of multiple sensors and ELISA-like systems in a very small space. It also possesses the potential to fabricate sensors on 3D surfaces and in otherwise hard-to-reach places inside channels. The presented method is investigated for step-coverage, deposition on non-planar surfaces, resolution limiting factors and durability.

#### **INTRODUCTION**

Protein patterning methods have attracted wide attention over the last two decades. Among the many presented methods, a photo-activated deposition approach may be the strongest candidate for fabrication of multi-sensor systems. However, reported photo-activated deposition methods require custom-made linkers or UV lasers to anchor proteins to the surface [1-2]. Photobleaching-assisted protein deposition, first shown by Holden and Cremer, eliminates those obstacles, being a verv straightforward method. They showed protein patterns deposited using a photomask where the labeled biotin is photobleached in the clear field using UV-lamp exposure and subsequently bonded to the substrate [3]. Later, Belisle et al. produced protein gradients using laser exposure for photobleaching [4]. They used a scanning laser and formed protein gradients by varying the exposure time.

Photobleaching-assisted deposition shown by Holden and Cremer is analogous to the photolithography used in microfabrication. Similarly, laser-activated deposition can be seen as the color photolithography of protein deposition. Using light at different wavelengths enables photobleaching of different dyes in different regions. It possesses great potential to form multi-sensor platforms in a region as small as a disk with a diameter of tens of  $\mu$ m. Such multi-sensor platforms can be deposited on non-planar surfaces, inner surfaces of microchannels and otherwise-hardto-reach places using the laser activated deposition.

This paper presents a set of experiments for laseractivated protein deposition to study step-coverage, resolution limiting factors, deposition on non-planar surfaces and durability. Lastly, an IgG sensor is fabricated using the aforementioned approach to demonstrate its feasibility.

#### **THEORY AND FABRICATION**

The protein deposition process has two steps; deposition of biotin and immobilization of streptavidin. A schematic view of protein deposition, including the sensor, is shown in Figure 1. First, fluorescently labeled biotin, biotin-4-fluorescein (Invitrogen, CA), is exposed to the laser illumination such that the fluorescent label is photobleached creating free radicals. Biotin is anchored to the surface when those radicals bind to the bovine serum albumin (BSA) (Invitrogen, CA) coated substrate. Then, streptavidin is immobilized through the avidinbiotin binding. This structure constitutes a platform for the realization of various biochemical immunosensors, hamster IgG sensor in this case. Biotinylated anti-hamster IgG (Southern Biotech, AL) is immobilized through the avidin-biotin binding completing the sensor fabrication. The sensing mechanism solely depends on the binding between the capturing molecule (anti-hamster IgG) and the target molecule (hamster IgG). The read-out is performed by fluorescence imaging of the captured molecule.

The spatial resolution of the method is determined by the diffraction limit of the laser optics and the diffusion length of the radicals.



Figure 2: Patterns of protein created by laser photobleaching. Left: Rectangular region with  $18 \ \mu m \times 70 \ \mu m$ . Right: Circle (bottom) and a square with rounded corners (top).

Thin micro cover glass slides with a PDMS barrier were used as a substrate. BSA was added for 20 min and followed by biotin-4-fluorescein. A Zeiss LSM-510-NLO confocal microscope with an argon laser at 488 nm was employed both for photobleaching and imaging. The substrate was illuminated between 30 s and 16 min depending on the size of the deposition area. After photobleaching, the chamber was rinsed with phosphate buffer saline (PBS) (Invitrogen, CA) and incubated with Alexa Fluor 633 (Invitrogen, CA) labeled streptavidin for 10 min. Then, it was rinsed with PBS and imaged with a Zeiss confocal microscope. For sensor deposition, the chamber was incubated with biotinylated goat anti-hamster IgG for 10 min after streptavidin deposition. Then, the chamber was rinsed with PBS and Alexa Fluor 488 labeled hamster IgG (Invitrogen, CA) was used to test the sensor response.



Figure 3: Array of protein squares to investigate the effect of power and exposure duration. Top: Array in a well. Each row has a different exposure time and each column has a different exposure power. Dashed arrows show when 2  $\mu$ l of liquid is added in order to keep the chamber wetted. Exposure before liquid addition is run with a lower liquid height. Bottom: Array in a microchannel where the liquid height is kept constant at 75  $\mu$ m. Longer exposure times are considered to give better results.





Figure 4: Images showing the step coverage of 36  $\mu$ m height difference. The top figure shows the locations of the stripes in images 1-10 with respect to the exposed region. The rectangle containing red and yellow stripes on the inclined surface shows the protein deposition area and red stripes in that area represent the stripes in images 1-10. In each imaging step from 1 to 10, the stage is raised by 4  $\mu$ m. The first image shows the deposition on the top surface. The following images demonstrate the deposition on the inclined surface.

#### RESULTS

Fluorescent images of rectangular and circular streptavidin patterns are shown in Figure 2. An array of different power levels and exposure durations was studied both in a well and in a microchannel, as shown in Figure 3, to observe limitations on pattern resolution. Depositions in an open chamber, like a well, showed varying resolution depending on the liquid film thickness. As the liquid layer becomes thinner, the resolution becomes better. This can be explained by the dispersion of free radicals after photobleaching. The exposed regions have 3D geometry, the third dimension being the liquid film thickness. As the thickness, the exposed

volume and the amount of photobleached particles increase. This contributes to the particle dispersion. Moreover, it may be also assumed that the increased volume will increase the thermal capacity increasing the time needed to reach thermal equilibrium. So, the convection flow will be effective for a longer time. When thickness is kept constant, as in the microchannel, lower power and longer exposure result in a better resolution.

Figure 4 shows the protein deposition on a non-planar surface and the coverage of a 36  $\mu$ m step. The laser was focused on the top surface and then the middle of the inclined surface for photobleaching. Acquired images show stripes at different heights. At each imaging step, the microscope stage is raised by 4  $\mu$ m moving the focal plane towards the bottom surface. Stripes are observed instead of a continuous rectangle because only fluorescence close to the focal plane is detected in confocal microscopy. From the superposition of presented images, it can be concluded that the pattern is continuous on the inclined surface.

Protein deposited samples were kept in PBS at 6°C to investigate their durability for 21 days. None of them presented any noticeable change in the coated area. In order to observe the effect of shear stress, patterns of protein were deposited in a microfluidic channel with a cross-section of 75  $\mu$ m × 900  $\mu$ m and exposed to fluid flow at various rates as shown in Table 1. No deterioration of the protein film could be observed.

Table 1: The change in protein area with fluid flow rate. The pattern is imaged after each fluid flow experiment and the measured area is compared to the initial area to delamination. Shear stress is calculated using  $\tau=6Q\mu/(wh^2)$ ; *Q*: flow rate,  $\mu$ : viscosity, *w*: width of the channel and *h*: height of the channel.

Fluid flow	Shear stress	Area/Reference
rate [ml/min]	[dynes/cm <sup>2</sup> ]	area
1.93	378	0.99
2.85	558	0.98
3.95	774	0.99
5.03	986	1.00
6.92	1360	1.03
1.24	2420	1.03

An IgG sensor is built in a microchannel to demonstrate the use of the method for bio-chemical sensing. A square pattern of streptavidin is formed and the biotinylated anti-hamster IgG is immobilized on that pattern. Figure 5 shows the fluorescent images of the pattern before and after the addition of labeled hamster IgG. Fluorescent images taken at 488nm after the protein deposition show that deposited biotin was photobleached. Thus, the fluorescent signal at 488nm after IgG dispension has no contribution from biotin-4-flourescein. The streptavidin patterns on the second row have better contrast compared to the images after IgG dispension. This shows that the sensor suffers from non-specific binding of the receptor or target molecule to the surface even if the protein deposition is highly specific.



Figure 5: Detection of AlexaFluor 488 labeled hamster IgG using the biochemical sensor. First row: After protein deposition, before IgG dispension (excited at 488 nm). Second row: After protein deposition, before IgG

dispension (excited at 633 nm). Third row: After IgG

dispension (excited at 488 nm). Scale bars are 20 µm.

Three sensors, presented in each column, are built with the same fabrication parameters (power, exposure duration, pattern size) but at different liquid-film thicknesses. Thickness is changed by the dispensed B-4-F solution volume. The volume used for sensor 1 is twice the one used for sensor 2 and four times the one used for sensor 3. The thickest one (sensor 1) gives a more powerful signal while showing a low pattern resolution. On the other hand, the thinnest one (sensor 3) gives a better resolution and lower detection signal. Three sensors fabricated with the same parameter but at different liquid thicknesses show a trade-off between the pattern resolution and the signal strength. The change in the amount of photobleached particles with increasing liquid thickness is discussed on the previous page. This might be the reason for the observed trade-off.

### DISCUSSION

Results show that patterns of proteins can be deposited on planar and non-planar surfaces with good step coverage. However, the method requires further investigation to determine the relation between the fluid thickness and pattern resolution. The deposited patterns are good for at least 21 days and can stand shear stresses larger than the blood circulation shear stress [5]. An IgG sensor is also fabricated to demonstrate the feasibility of the method.

The proposed method is promising for fabrication of ELISA-like multi-sensor systems in small regions and even in microchannels. It also enables fabrication of sensor systems in a 3D geometry further increasing the sensor packaging capacity. Thus, laser-activated protein deposition can have various applications in lab-on-chip systems, microfluidic diagnostic tools and implantable devices.

# ACKNOWLEDGEMENT

This work was supported by the Center of Excellence in Genomic Science: Biologically Active Microsystems, National Institutes of Health, National Human Genome Research Institute, grant 5-P50-HG002360-06 and Nanotechnology Graduate Research Award from the University of Washington Initiatives Fund (UIF).

## REFERENCES

1. D. N. Adams, E. Y.-C. Kao, C. L. Hypolite, M. D. Distefano, W.-S. Hu, and P. C. Letourneau, "Growth cones turn and migrate up an immobilized gradient of the laminin IKVAV peptide", Journal of Neurobiology, 62 (1), pp.134-147, 2005.

2. A. Buxboim, M. Bar-Dagan, V. Frydman, D. Zbaida, M. Morpurgo, and R. Bar-Ziv, "A single-step photolithographic interface for cell-free gene expression and active biochips", Small, 3 (3), 500-510, 2007.

3. M. A. Holden, and P. S. Cremer, "Light Activated Patterning of Dye-Labeled Molecules on Surfaces", Journal of the American Chemical Society. 125 (27), 8074-8075, 2003.

4. J. Belisle, J. Correia, P. Wiseman, T. Kennedy, and S. Costantino, "Patterning protein concentration using laser-assisted adsorption by photobleaching, LAPAP", Lab on a Chip, 8 (12), 2164-2167, 2008.

5. C. P. Cheng, D. Parker, and C. A. Taylor, "Quantification of Wall Shear Stress in Large Blood Vessels Using Lagrangian Interpolation Functions with Cine Phase-Contrast Magnetic Resonance Imaging", Annals of Biomedical Engineering, 30 (8), 1020-1032, 2002.